

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



A TOYOTA MOTOR CORPORATION INVENTION  
PCT/US01/10982

(43) International Publication Date  
11 October 2001 (11.10.2001)

PCT

(10) International Publication Number  
WO 01/74161 A2

(51) International Patent Classification<sup>7</sup>: A01N 25/04,  
25/30, 63/04, 63/00, 43/16 // (A01N 63/04, 25:30, 25:04)  
(A01N 63/00, 25:30, 25:04) (A01N 43/16, 25:30, 25:04)

(21) International Application Number: PCT/US01/10982

(22) International Filing Date: 4 April 2001 (04.04.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/194,573 4 April 2000 (04.04.2000) US  
60/218,968 17 July 2000 (17.07.2000) US

(71) Applicant: ABR, LLC [US/US]; Suite 1610, 1188 Bishop  
Street, Honolulu, HI 96813 (US).

(72) Inventor: HIROMOTO, Bryan; 295 Hiwalani Loop,  
Pukalani, HI 96768 (US).

(74) Agents: MURASHIGE, Kate, H. et al.; Morrison & Foer-  
ster LLP, Suite 500, 3811 Valley Center Drive, San Diego,  
CA 92130-2332 (US).

(81) Designated States (*national*): AE, AL, AM, AT, AU, AZ,  
BA, BB, BG, BR, BY, CA, CH, CN, CO, CR, CU, CZ, DE,  
DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID,  
IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT,  
LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL,  
PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ,  
UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian  
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European  
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,  
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,  
CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published:**

— without international search report and to be republished  
upon receipt of that report

*For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.*

(54) Title: IMPROVED PESTICIDE MICROEMULSIONS AND DISPERSANT/PENETRANT FORMULATIONS

(57) Abstract: A clear aqueous emulsion of a complex comprising a moiety having a hydrophilic core to which is covalently bound the backbone of at least one monounsaturated C<sub>16</sub>-C<sub>20</sub> fatty acid and appropriate surfactants is effective in controlling agricultural pests, including nematodes.

WO 01/74161 A2

## IMPROVED PESTICIDE MICROEMULSIONS AND DISPERSANT / PENETRANT FORMULATIONS

### Technical Field

The invention relates to agricultural pest control. More specifically, the invention concerns use of natural product derived microemulsions to control agricultural pests, in particular plant nematodes.

### Background Art

The negative impact of infestation by pests on agricultural production is well-known. Damage worldwide due to nematode infestation alone is estimated at \$78 billion per year. Other sources of infestation include bacterial, fungal, and insect pathogens. Assault on these infestations has taken the form of use of synthetic pesticides derived from petroleum, use of natural predators, and use of compounds derived from natural sources, such a pyrethrins. No completely satisfactory pesticide which is specific, effective, and not harmful to the environment has been found. Thus, there is a considerable need for finding improved pesticides.

With regard to previous compositions related to those of the present invention, the effect of fatty acids on a particular species of plant nematodes (*Meloidogyne javanica*) has been described by Sitaramaiah, K., *et al.*, *Indian J. Nematol.* (1977) 7:58-65. Formic, acetic, propionic and butyric acids were tested. The effect of a collagenolytic/proteolytic enzyme secreted from *Bacillus cereus* after induction by collagen on this plant nematode species has also been reported by Sela, S., *et al.*, *Eur. J. Plant Pathol.* (1998) 104:59-67. The enzyme was effective to digest collagen from cuticles of second stage juveniles. U.S. Patent 5,698,592 describes compositions for the control of plant infective nematodes using fatty acid compounds which compositions are microemulsions containing C<sub>8</sub>-C<sub>14</sub> fatty acid esters. The esters were prepared synthetically and the nature of the microemulsions is not described.

Fatty acid or oil compositions have also been described for other purposes, including the use of a vegetable oil/mineral salt broth fermented by yeast as an emulsifier in the food industry, as described in U.S. Patent 4,810,507; the use of a mixture of an oleate derivative, a lower alkanol, and an inert diluent as a pharmaceutical penetration agent, as

described in U.S. Patent 4,863,970; and the use of a solution of oleic acid, acetone, and ammonium hydroxide in water as a treatment for athlete's foot, as described in U.S. Patent 4,172,888. Unsaturated lipid compounds containing polar head groups, including oleates, are described as cell-envelope disordering compounds.

The use of microbial infection to control nematode infestation in plants was described in U.S. Patent 5,264,210. PCT Application W098/59036 describes compositions for the control of nematode infestation using metabolites from fungi. In some cases, the fungi are cultured in media which include vegetable oil and the compositions may include surfactants.

U.S. patents 5,674,897; U.S. 5,698,592; and U.S. 6,124,359, all assigned to Mycogen describe microemulsions containing fatty acid esters for use as nematocides. These fatty acid esters are of C<sub>8</sub>-C<sub>14</sub> fatty acids and they do not appear to include glyceride esters. Various other patents disclose the use of alkoxyated materials and other emulsifiers for use in pesticides or herbicides intended for application to plants. These include U.S. patents U.S. 4,975,110 and U.S. 5,098,467 to Safer; U.S. patent 5,827,522 to Troy and U.S. patent 6,093,681 to Monsanto. In addition, U.S. patent 5,362,707 assigned to Rhone Poulenc describes the use of "suspoemulsions" as vehicles to apply pesticides with solid active ingredients (having melting points >45°C) to plants.

It has not heretofore been appreciated that complexes containing a hydrophilic core, at least one monounsaturated fatty acid backbone, surfactants, and including a polyalkoxylene, (such as compositions containing a denatured lipase with long chain fatty acids or their derivatives) prepared in the form of a clear microemulsion are particularly effective in controlling pests in agricultural settings.

In addition, the formulation of agents employed for dispersal and penetration of active ingredients is useful as a carrier for plant nutrients, pesticides in general, herbicides, and other materials suitable for application to plants and is also useful as a topical drug delivery system.

A wide variety of carriers for active ingredients has been devised in both the pharmaceutical and agricultural arts. For example, drug delivery systems have included various forms of liposomes, various polymeric slow-release compositions, detergents which aid in penetration, and other excipients that affect the behavior of small molecules or proteins or other biological molecules that are considered to be the active ingredients. The

formulation of the present invention, originally developed as a carrier for nematocides, has been found useful as a delivery system for pesticides in general, for plant nutrients, and for topical application of drugs as well. The carrier of the present invention is particularly effective in dispersing active ingredients over a wide area and in effecting penetration of these active ingredients through cell walls and membranes and is thus useful both in agricultural and pharmaceutical contexts.

#### Disclosure of the Invention

The invention is directed to environmentally friendly but effective pesticides for use on plants. The pesticide can be prepared in a premixed concentrated form and then diluted for application to agricultural crops such as tomato, potato, pineapple, and any other target crop which is subject to pest infestation. The composition is a microemulsion that appears clear to the naked eye. Indeed, the turbidity of the microemulsion must be quite low - when measured with a turbidometer, the turbidity should be less than about 3 NTU, preferably less than 2.5 NTU, more preferably less than 1 NTU. The clear microemulsion will contain as active ingredient a complex which comprises a moiety having a hydrophilic core to which is covalently bound at least one backbone of a monounsaturated fatty acid of C<sub>16</sub>-C<sub>20</sub>, preferably an oleic acid backbone. As used herein, "backbone" of a fatty acid refers only to the carbon chain and does not include the carboxyl group oxygens. Typically, this core is a saccharide, such as sucrose, glucose, or a more complex saccharide esterified with one or more oleic acid or similar residues. The complex moiety may also be formed from the association of a denatured lipase and a long chain monounsaturated fatty acid or its esters. The complex will also contain detergents and some form of polyoxyalkylene, such as polyoxyethylene. The polyoxyalkylene may be associated directly with the moiety described above or may be included in a separate portion of the complex.

Thus, in one aspect, the invention is directed to the active ingredient of the pesticide, which is a complex consisting essentially of at least one C<sub>16</sub>-C<sub>20</sub> monounsaturated fatty acid or ester thereof associated with a denatured lipase, an anionic surfactant blend, a microemulsion surfactant blend, and polymers which are polyoxyalkylene polymers. The polyoxyalkylene is supplied by providing alkyoxylated linear alcohols of C<sub>9</sub>-C<sub>11</sub>. In another embodiment, the active ingredient is a complex consisting essentially of a saccharide esterified with at least one monounsaturated C<sub>16</sub>-C<sub>20</sub>

fatty acid and the anionic surfactant blend, a microemulsion surfactant blend, and polymers which are polyoxyalkylene polymers. The polyoxyalkylene polymers are preferably provided by derivatizing the saccharide.

In another aspect, the invention is directed to a pesticide premix consisting essentially of a homogenized mixture of the components set forth above. Further, the invention is directed to the diluted form of the premix and to a method to control agricultural pests which comprises applying the pesticidal dilution to an agricultural crop.

The invention is further directed to methods to prepare these pesticide compositions.

Specifically, the carrier comprises at least three components. The first component is at least one linear alcohol of 7-12C, preferably 9-11C, which is polyalkoxylated. The second component is at least one surfactant which is designed to promote formation of microemulsions. The third component is at least one anionic surfactant. If desired, a disproportionate amount of a linear alcohol of 11-12C with a high degree of polyalkoxylation may also be included. The components should be mixed in a particular order in order to ensure maximum effectiveness.

Thus, in another aspect, the invention is directed to a dispersant/penetrant which consists essentially of:

- at least one linear alcohol of 7-12C which linear alcohol is polyalkoxylated;
- at least one surfactant that promotes microemulsion; and
- at least one anionic detergent.

The invention is also directed to agricultural and pharmaceutical compositions which comprise at least one active ingredient (for example, the nematocide complex described above) and the dispersant/penetrant compositions of the invention. In still another aspect, the invention is directed to methods to prepare and use these formulations.

#### Modes of Carrying Out the Invention

The pesticide compositions of the invention are applied to crops or to fields or environments where pesticide control is desired in the form of clear microemulsions. The clarity appears to be important. Not only does the final composition need to appear clear to the naked eye, it should have a very low turbidity index when measured in a turbidometer. Preferably, the composition should be less than 3 NTU, more preferably less than 2.5 NTU, and still more preferably less than 1 NTU. Pure water has an NTU value of 0.3.

The compositions ultimately to be applied are prepared by diluting a premix which contains the active ingredient.

While not intending to be bound by any theory, it is believed that the active ingredient in the pesticide compositions in the invention is a complex comprising a moiety containing a lipophilic component and a hydrophilic portion which can be associated with an anionic surfactant. There are two embodiments of the active ingredient which share these characteristics.

#### Lipase/Fatty Acid Complexes

In one embodiment, lipases which can complex with triglycerides comprising fatty acids which contain a single double bond and which have maximal activity on fatty acid chain lengths of 8 carbons are employed. These have minimal enzymatic effects on esters of C<sub>18</sub> fatty acids. In a preferred embodiment, the fatty acids which are present in the complexes of the present invention may have chain lengths of 16-20 carbons. More preferably, the chain length of the triglyceride-esterified fatty acid used in the composition is C<sub>18</sub>. The lipase may merely associate with the triglyceride and not hydrolyze it; indeed, in some compositions of the invention prepared from oils comprised primarily of oleate triglycerides, compositions which contain lipases derived specifically from *Laetiporus sulphureus* contain the same level of triglyceride as untreated oils. Moreover, lipases suitable for the compositions of the invention associate not only with triglycerides, but also with diglyceride and monoglyceride and other esterified forms of the fatty acids as well as with the free fatty acids themselves, provided they include fatty acids of 16-20, preferably 18 carbons in length.

Many of such lipases are of fungal origin. Since triolein is the primary storage oil of many fungi, certain fungal lipases are genetically designed not to degrade the naturally occurring triolein in the environment, but catalyze the degradation of other triglycerides with different fatty acid chain lengths. This would result in intact triolein, diolein, monolein and oleic acid in the environment of a fungal colony producing this type of lipase enzymes on a mixed fatty acid source. This type of lipase is relatively specific for 8-carbon fatty acids and will only hydrolyze C<sub>18</sub> fatty acids to a limited extent. The enzyme attaches to the triglyceride containing oleic acid, recognizes the chain length, and releases the triglyceride only when suitable cleavable triglyceride is available. If not, the lipase will remain attached to the uncleavable triglyceride. Thus, when these lipases are coupled with

a triglyceride consisting of  $C_{18}$  fatty acids, the lipase will attach, but not cleave the fatty acids from the glycerol.

It will be noted that in this embodiment, the hydrophilic core includes the oxygens of the carboxyl groups of the fatty acid residue; thus, the "backbone" of the fatty acid is covalently bound to the hydrophilic core, which includes these oxygens as well as the lipase.

In order to stabilize the complex, it is preferred that the lipase portion of the complex be denatured. Thus, the complex, after formation, is treated, preferably by heating to a suitable temperature, to denature the lipase protein.

The complex can be prepared by utilizing purified preparations of suitable lipases preferably but not necessarily derived from fungal sources and permitting the lipase to associate with a fatty acid or fatty acid ester wherein the fatty acid backbone has the appropriate length ( $C_{16}$ - $C_{20}$ ) and the preferred amount of unsaturation (one double bond). The ester may be an ordinary ester such as a methyl, ethyl, ethylene glycol, propyl, or butyl ester, and the like, or, preferably, the fatty acid is esterified to glycerol either as a mono, di and preferably triglyceride. Triglycerides are readily available as components of vegetable oils, and thus vegetable oils constitute a preferred source, especially oils that contain high concentrations of trioleate, such as safflower or sunflower seed oil.

The complex, for use in the compositions of the invention must be denatured. The denaturation step is typically performed by heat, but other denaturing agents, such as chaotropic agents and adjustment of pH could also be used. The denaturation step is an essential one in preparing the resultant hydrophobic/hydrophilic complex.

Thus, for preparing these compositions, a vegetable oil, high in trioleate content is preferred. Such an oil is, for example, Optimum™ Sunflower Oil, Optimum Quality Grains, Inc., Urbandale, IA. Other oil compositions which contain mono, di, or triglycerides of fatty acids of suitable length may also be used. The presence of a single double bond in the fatty acid is preferred. It should be emphasized that the trioleate must contain at least one monounsaturated fatty acid of 16-20 carbons in length, preferably 18 carbons, but this need not be the only fatty acid component of the oil. As long as substantial amounts of this fatty acid are present.

Oil sources include nonplant sources such as from fish or whales which would increase the carbon chain possibilities for monounsaturates from a range of  $C_{16}$  -  $C_{20}$  to  $C_{16}$  -  $C_{26}$ . For forming the complex, suitable lipases can be obtained from plant, fungal,

bacterial, and animal sources. One suitable lipase is wheat germ lipase. (Wheat germ lipase does not digest triolein.) Suitable fungi include *Laetiporus sulphureus*, *Ganoderma spp*, *Mucor spp*, *Rhizopus spp*, and *Penicillium spp*. Also available are lipases from fungi such as *Candida spp* and *Aspergillus spp*. Suitable bacteria include *Pseudomonas spp*, *Rhizobium spp* or *Chromobacterium spp*. Suitable animal sources include pigs, goats, sheep, and bovine sources. Lipases are also commercially available such as those obtainable from Valley Research Technologies, South Bend, IN, which markets Validase 8000, a lipase commonly used in cheese-making. Of course, mixtures of individual lipases can be used. Typical lipases useful in the invention are those described by enzyme No. ECC 3.1.1.3 or CAS No. 9001.62-1.

A wide variety of lipase sources can thus be used. One such source is cheese itself and the various microorganisms used in its manufacture. It should be emphasized that because many cheeses have a high lipase content, such cheeses can be used in the formulation *per se*. The types of cheese that can be used constitute a wide variety, including cottage cheese, cream cheese, brie, mozzarella, Monterey Jack, Munster, blue cheese, cheddar, edam, gouda, Swiss, parmesan and romano. Particularly preferred is chevre which has a high moisture content and appears to be very high in lipase content, especially when fresh. Blue cheese and brie also have high contents of lipase. The bacteria and fungi that are used in the manufacture of these cheeses also are good lipase sources. These microorganisms include, prominently, *S. lactus*, *S. cremoris*, and *S. thermophilus*.

In addition to these microorganisms, the ATCC web site provides an extensive list of microorganisms including many *penicilia* and *kluyveromyces* that produce lipase. There is an extensive list of fungi in general, and yeast in particular that produce lipase that is useful in the invention. Such organisms include *Aspergillus oryzae*, *Aspergillus soyae*, *Sachromyces rouxii*, *Aspergillus flavus*, *Hansenula subpelliculosa*, *Zygo- sachromyces*, *Lactobacillus delbraeki*, *Rizous oligosporus*, *Neuraspura sitophila*, and many others. In addition, a wide variety of yeasts, as listed in the American Type Culture Collection Catalog, current edition, incorporated herein by reference, lists multiple yeast genera which can be used as sources of lipase.

Finally, lipases are found in seeds of germinating plants, such as wheat, oats, corn, rice and rye, as well as plants where seeds contain substantial amounts of triglycerides, such as soy beans, safflower, canola, corn, cotton seed, sunflower, olive, walnuts, macadamia nuts, peanuts, and the like can be used as a source of lipase. Crude extracts can



be used or the lipase can be purified to any desired extent. In these preparations, other stabilizing components, such as dextrin, may be included.

In general, the ester and lipase are mixed together in an aqueous environment for a sufficient time and at an appropriate temperature to permit complex formation. Typically, complex formation occurs over a period of several hours at slightly elevated temperatures. Typical conditions would be represented by periods of 10-40 hours at temperatures of 30-50°C. Then the oil phase is recovered and subjected to treatment to denature the lipase, typically treatment using heat. Denaturing conditions vary depending on the components utilized, but typically such conditions would include treatment at 90-99°C for 5-20 minutes. In one manner of performing this step, the lipase is purified.

In one embodiment, to prepare the lipase/fatty acid or ester complex, above, the lipase may be supplied in the form of a culture filtrate from cultivation of a suitable fungus, such as those set forth above as suitable sources for the lipase. In this approach, the fungus is cultivated for a period of 10-30 days, preferably around 20 days, in a cultivation vessel forming a mycelium mat. Suitable nutrients are those typically appropriate for the cultivation of the particular species of fungus employed. After the incubation period, the culture fluid is separated from the mycelium. The separation may occur when the fungus is "immature" i.e., prior to the termination of growth phase or when the fungus is "mature" - i.e., at the point where growth phase begins to level off. The culture fluid is filtered and then mixed with the oil (or other fatty acid ester) for an appropriate time period, such as 18-30 hours, preferably around 24 hours at 27-35°C, preferably 33°C. After this incubation period, the oil phase is separated and the enzyme complex is denatured preferably by heat. Typical times and temperatures are 20 minutes at 100°C or until the emulsion clears. Any particles which remain are filtered off.

When a purified lipase is used, an aqueous solution of the lipase is mixed with a suitable oil and the mixture is homogenized using stirring at high speed (i.e. 30,000 rpm) or other means of homogenization such as mixing in a blender or sonication. A Power Gen Homogenizer Model 1700 with a variable speed range of 10,000 - 30,000 rpm is available from Fisler Scientific. In this step, the ratio of the lipase or lipase mixture to the oil is important. If Validase 8000 fungal lipase is employed, 2 gm of the enzyme is mixed with 600 ml of water and stirred for 1 hour. The resulting enzyme solution is then added to 1 liter of sunflower oil, high oleic type, Optimum Quality Greens, LLC, Urbandale, IA, in a typical procedure. As set forth above, it appears that an optimum condition is a simple

complex between the triglyceride and the lipase. The homogenized mixture is then incubated for a time and at a temperature sufficient to assure the association of the fatty acid or fatty ester components of the oil with the lipase. Typically, the incubation period is from 10-30 hours, preferably 15-27 hours, most preferably 24 hours and the temperature is elevated above room temperature, preferably 25-50°C, more preferably 30-45°C, and most preferably 40°C. Optimum conditions appear to be 24 hours at 40°C. Alterations of the incubation time and temperature are, however, possible since the time and temperature factors are interdependent.

After a suitable incubation period, the oil phase is separated and treated to denature the enzyme and stabilize the complex. Typical denaturation procedures include heat, typically at 95°C for about 10 minutes. Other denaturation procedures may, however, be used, such as microwaving, pH adjustment and chaotropic agents. During heat denaturation, the emulsion clears. The denaturation is necessary to prevent enzymic activity with respect to the linear alcohols that will be added later. Heat denaturation also removes water from the lipase structure. Water interferes in the surfactant addition performed later in the formulation. Further, the lipase will no longer be able to attach to new substrate. The emulsion separates into an oil and water bi-layer; the oil layer is retained and the water layer discarded. The water layer does not form an emulsion with new oil due to the heat denaturation process.

Once the denatured complex is stabilized, any solid particles that are present are removed by centrifugation or filtration or any other effective procedure. There may be no solid particles in some instances. Surfactants are then added to complete the formation of the active complex.

#### Saccharide/Fatty Acid Molecules

In another embodiment of the active ingredient, the complex comprises a saccharide, such as sucrose, sorbitol, xylitol, glucose, raffinose, or erythritol esterified to at least one fatty acid residue which is monounsaturated and contains 16-20C, preferably 18C (*i.e.*, oleic acid). The saccharide core may be esterified to one or more than one, such as 2, 3, 4 or 5 fatty acids. At least one of the fatty acids must be of the appropriate size and unsaturation; as long as this is the case, other types of fatty acid backbones may also be included. It is preferred that the polyol saccharide cores be also derivatized with polyoxyalkylenes, in particular, polyoxyethylene. In addition to saccharide cores, other

alcohols may also be used, such as glycerols, which may contain polyoxyalkylene chains as well. For example, ARCO Chemicals has commercialized esterified propoxylated glycerols. The Olestra™ products of Procter and Gamble are sucrose polyesters containing fatty acids in the range of 8-22 carbons; to the extent that oleates or monounsaturated fatty acids in the C<sub>16</sub>-C<sub>20</sub> range are included among these, these moieties are also useful in the invention. Particularly preferred is the commercially available material Toximul SEE-340, a sorbitan trioleate ethoxylate containing 20 moles of ethylene oxide. It may be advantageous, in this instance, to solubilize the Toximul SEE-340 initially in a mixture of aromatic hydrocarbons such as Exxon Aromatic 100. Other sorbitol esters and sorbitol ester ethoxylates can also be used. These are commonly referred to as SPANs, TWEENS, sorbac and polysorbac.

#### Addition of Surfactants

While not intending to be bound by any theory, in both embodiments above, it is believed that the long chain hydrophobic portion of the complex penetrates the lipid layers of the cells or membranes of the target pest, while the hydrophilic portion, preferably associated with an anionic detergent, becomes attached to the proteins embedded in the lipid layers. The effect is believed to be enhanced by the presence of shorter chain alcohols. Further, suitable detergents and ethoxylated alcohols permit an effective microemulsion and effective dispersion of the complex for application. In compositions where the active ingredient is a lipase associated with a fatty acid or derivative, polyoxyalkylene polymers are advantageously added in the form of the above-mentioned ethoxylated alcohols. This can be done in any composition of the invention. However, in those compositions where the moiety comprises a saccharide core esterified with fatty acids, polyoxyalkylene polymers may be derivatized directly to the core. In any case, a preferred form of polyoxyalkylene is polyoxyethylene.

Regardless of the nature of the active ingredient used, addition of surfactants is required in order to complete the effective complex. It appears that the order of addition of surfactants is important. In the case of hydrophobic/hydrophilic moieties where a polyoxyalkylene is already included, further addition of such polymers may be unnecessary. However, in the case of embodiments where the hydrophobic/hydrophilic moiety is a complex with denatured lipase, it is often necessary or desirable to include a source of such polyoxyalkylene polymers. Inclusion of additional such polymers may also

be desirable even though the hydrophobic/hydrophilic moiety contains some level of such entities. In these cases, initially, an alkoxyated, typically ethoxylated, linear alcohol or alkoxyated/ethoxylated mixture of alcohols containing 9-11 carbons is added followed by homogenizing. This is optionally followed by adding an ethoxylated linear alcohol of 11 carbons, and then homogenizing. This is then followed by adding a surfactant blend for microemulsion formation and homogenizing, and finally an anionic surfactant is added. The final composition is then homogenized. By using appropriate concentrations, the resultant forms a concentrated premix which then can be diluted for application to agricultural crops.

Thus, in the first step described above, mixtures of linear alcohols of suitable chain lengths with an appropriate degree of alkoxylation can be used. The proportions of C<sub>9</sub>, C<sub>10</sub> and C<sub>11</sub> alcohols can be variable, but typically, the mixture contains at least 25 percent of C<sub>9</sub> linear alcohols. Commercially available mixtures are typically ethoxylated to varying degrees, 2.5 moles of ethoxylation in the polymeric chain being preferred. Although ethoxylation is the most typical commercially available form, there is no theoretical reason that methoxylation or formation of polymeric ethers with higher chain alcohols could not be used. Tomah Reserve, Inc. markets a series of linear alcohols trademarked Tomadol<sup>TM</sup>. The Tomadol<sup>TM</sup> compositions vary in chain length of the alcohol and number of moles of ethoxylation. For compositions where the alcohol contained 11 carbons, 7 moles of ethoxylation per mole of alcohol appeared optimal; 9 moles resulted in a solid at room temperature. Further, in compositions which contain mixtures of 9-11 carbon alcohols, 2.5 moles ethoxylation per mole of alcohol appeared to exhibit the best insect cuticle penetration.

Similarly, in the second (optional) step, C<sub>11</sub> linear alcohols in ethoxylated form are also added. Typical levels of ethoxylation include 7 moles of monomer in the polymeric chain; as was the case with the ethoxylated mixture in the first step, other alkoxyated forms could be substituted.

In the third step, a surfactant blend for microemulsion formation is employed. Various commercial preparations are available, including those from Stepan Chemical Company, Winder, Georgia. Of seven microemulsion formulations produced by Stepan Chemical, the highly preferred formulation is commercially designated H306A. The company will produce this formulation on special order of 20,000 lb. batches.

Microemulsion concentrate formulations such as are described by Skelton, P.R., "Pesticide Microemulsion Concentrate Formulations Utilizing Fatty Acid Methyl Esters as Solvent Alternatives," *Pesticide Formulations and Application Systems: 13th Vol., ASTM STP 1183*, Paul D. Berger, Bala N. Devisetty, and Franklin R. Hall, Eds, American Society for Testing and Materials, Philadelphia, 1993. Components can include combinations of the following: methyl esters, sorbitan ester ethoxylates, butoxy block copolymer, alkyl benzene sulfonic acid, and calcium salt. Alternatives for the ethoxylate and/or the block copolymer can be castor oil ethoxylates. The foregoing are only examples, and the formulations are not limited to those components. Other microemulsion formulations are available.

In the fourth step, an anionic detergent or mixture of anionic detergents is used. Anionic surfactants, such as sodium lauryl sulfate and ammonium lauryl sulfate are known. In general, such surfactants include: alkylarylsulfonates, linear alkylbenzene sulfonates, alkane sulfonates, alcohol sulfates, alcohol ether sulfates, polyether carboxylates, olefin sulfonates,  $\alpha$ -sulfomonocarboxylic esters, sulfosuccinates, phosphorus-containing anionics, and phosphate esters. Commercial mixtures are also available, although the variety of options available has decreased due to the introduction of siloxane-based surfactants now more commonly used in agriculture. However, anionic detergents are important in the compositions of the invention for several reasons. First, the anionic surfactant is believed to attach to the remainder of the lipase with which the oil, such as triolein, is associated. Anionic surfactants would associate, also, with the hydrophilic core in the case of sugar-based moieties. The result is that the complex, bearing a negative charge, will be more effective in binding nematode membranes in the soil. Second, in the case of acidic soils, additional anionic surfactant is desirable to neutralize the positive charges in the environment. One anionic surfactant currently marketed is Atsurf 1910 which, in its newer version at surf 1910 HF with a higher flash point, is less desirable than the version previously sold.

Typically, the composition is diluted 1:200, 1:500, 1:1000, or 1:2000 for application. The final concentration of the fatty acid backbone component is on the order of 200 ppm. The diluted composition must be clear and not turbid. Initially, it is apparent that any dilution which does not appear clear to the naked eye should be rejected. It is preferable, however, that the turbidity of the final composition be less than 3 NTU, more preferably less than 2.5 NTU, and most preferably less than 1.0 NTU as measured in a

turbidometer. The dilution may result in a satisfactorily clear solution without further agitation, or it may be necessary to stir, shake or vortex or otherwise agitate the dissolved components in order to effect clarity. Application of the solution can be by conventional means such as irrigation, spraying, and the like. Suitable target crops are any that are affected by undesired infestation by bacteria, fungi or nematodes, in particular by nematodes. The composition has been extensively tested on pineapple.

In somewhat more detail, the premix is diluted with additional surfactant and emulsifier, if desired, to the requisite dilution. The dilution is determined by the extent of infestation, the nature of the crop, and its stage of development. After dilution to form an additional clear microemulsion as described above, the diluted material is applied to the surface of the plots containing the plants. If desired, observations of the effectiveness of the pesticide may be taken systematically. Such observations may include the health of the plant generally, or may also involve specific measurement of pest viability. As exemplified below, suitable dilutions were able to lower nematode viability on pineapple crops to a very low level.

Soil moisture levels are important. If the soil is water saturated, the solution will not penetrate the soil particles and effectiveness drops. Thus, if it rains and the soil becomes soggy, it is preferred to delay application. Similarly, if a large amount of water is added as a post wet treatment, the effects may be diluted. Rains after application may also affect effectiveness. Larger or more concentrated dosages will kill within 1 day, some of the stronger formulations will kill 100% in 45 minutes of exposure. Weaker formulations may take several days to reach the same effectiveness.

The particular choice of formulation and the level of dilution may also determine the level and type of possible side effects on plant growth and health. In some formulations on some plants, growth is enhanced in addition to effecting nematode control. In some cases, the formulations may be slightly inhibitory or toxic. In general, the preparations which include fungal digested oil in relatively high concentrations are best used on pineapples as target crops and work less effectively, when taken in terms of total effect, on tomatoes. However, these aspects are independent of the nematode control aspects of the formulation and the formulations and dilution levels can readily be optimized for any particular type of plant.

The more general carrier formulations of the invention are designed to assist dispersal and penetration of active ingredients such as pesticides or drugs. However, in

some instances, the invention compositions may themselves have applicability as pesticides where membrane disruption is the only requirement for effectiveness. Further, the compositions with or without active ingredients may be used as formulated, or, especially with respect to application to crops, may be diluted in water, often to a very high degree - *i.e.*, dilutions of 1:10, 1:100, 1:1,000 or 1:10,000 may be acceptable depending on the context. In all cases, for maximum effectiveness, the final formulation should appear clear to the naked eye. If the turbidity is measured with instrumentation, it is preferred that the turbidity be less than 5 NTU, more preferably less than 3 NTU, and most preferably less than 1 NTU.

The composition's three essential components can further be described as follows:

The first component is a linear alcohol containing 7-12C, preferably 9-11C which is derivatized with a polyalkoxylene moiety, such as polyethylene oxide. Of course, mixtures of such linear alcohols can be used. The variables in this component include the number of carbons in the linear alcohol and the number and nature of alkoxylenes in the polyalkoxylation. Generally, mixtures containing approximately equal amounts of alcohols containing 9-11 carbons is preferred, although a preponderance of C<sub>11</sub> alcohols, especially where the polyalkoxylene ratio is relatively high - *i.e.*, on the order of 5-9, may be included within this mixture or added subsequently. It is not necessary to include a mixture of such alcohols and a single, for example, C<sub>10</sub> alcohol could be used. The degree of polyethoxylation or polyalkoxylation is also acceptable within a range; in general, the majority of the alcohols should be polyalkoxylated in a ratio of 2-6 alkoxylenes units is preferred. For practical reasons, polyethoxylene is the preferred polyalkoxylene - thus it is possible, in a preferred embodiment, to consider the linear alcohols "PEGylated."

The degree of polyalkoxylation that is optimum for a particular composition will depend on the nature of the alkoxylenes in the polymer, the chain length of the linear alcohol, and the nature of the remaining polyalkoxylated alcohols in any mixture that might be used. In a typical formulation, a mixture of C<sub>9</sub>-C<sub>11</sub> linear alcohols at a PEGylation ratio of 2.5 moles of ethylene glycol per linear alcohol is used, supplemented with a C<sub>10</sub> or C<sub>11</sub> PEGylated alcohol at a ratio of 6-8, preferably 7 moles of ethylene glycol per linear alcohol unit.

The alkoxylation ratio describes the ratio of alkoxylenes units to linear alcohol. The ratio may represent an average - *i.e.*, not all of the molecules in the mixture may show the same ratio.

Polyalkoxylated alcohols suitable for inclusion in the compositions of the invention may be formulated independently or are commercially available. For example, Tomah Reserve, Inc. markets such mixtures.

The second required component is a surfactant or mixture of surfactants which promotes microemulsion formation. Typically, the surfactants included in this component are nonionic and amphiphilic. Microemulsion formation is apparently best promoted by mixtures of such surfactants. Suitable components of such mixtures include such nonionic surfactants as esterified phospholipids and sorbitol esters with long chain fatty acids. Suitable mixtures can be formulated independently and are also commercially available, for example, from the Stepan Chemical Co., Winder, GA.

The third component is an anionic detergent, such as the ammonium and metal salts of sulfonated fatty acids. Suitable anionic detergents include, for example, ammonium and sodium lauryl sulfate, aromatic sulfonic acid salts, and the like.

Optimum results in terms of encouraging dispersal and penetration are obtained when the components of the mixture are added in the order listed above with mixing after each step. Thus, the microemulsion-enhancing surfactant is added to the polyalkoxylated linear alcohol component(s) and thoroughly blended prior to the addition of the anionic detergent. In all cases, suitable agitation and mixing are required. The ratios of the components can vary, but it is preferred to include about 1-5 parts polyalkoxylated linear alcohol to about 9-15 parts microemulsion enhancer to about 9-15 parts anionic detergent. Particularly preferred ratios of components comprise about 5-7 parts polyalkoxylated alcohols to 11-13 parts microemulsion enhancer to about 11-13 parts anionic detergent. A particularly preferred ratio is 7:12:12.

The final form of formulation which includes the compositions of the invention depends on the nature of the application. For pharmaceutical applications and topical drug delivery, preferably the composition containing an active ingredient is applied in undiluted form. The active ingredient or active ingredients can be added directly to the composition or, alternatively, the composition can be built around the active ingredients. Any pharmaceutically active compound is an appropriate active ingredient, including small molecule pharmaceuticals, proteins, antiviral agents, antibiotics, anti-inflammatory agents, beta blockers, and the like. The reader is referred, for example, to the current edition of the Physician's Desk Reference, incorporated herein by reference.



For a pharmaceutical use, additional components may be added to the final formulation so long as they do not distort the dispersant/penetrant qualities of the carrier composition of the invention. For example, antioxidants, stabilizers, and salts for controlling pH may be added to the formulation. Small amounts of diluent may also be included.

For agricultural applications, although the compositions may be used *per se* as, for example, nematocides by virtue of their ability to disrupt membranes, typically, they are supplied with an additional active ingredient such as an herbicide, pesticide or nutrient. Because of the requirement to provide the active ingredient over a wide area, the final formulation is typically diluted. Quite large dilution ratios are frequently employed so that the active ingredient is present only in 100-500 ppm, for example. Typical active ingredients include cytokinins, auxins, gibberellic esters, indole acetic acid, and similar compounds. Preferred compounds include zeatin, gibberellic acid, and abscisic acid. Also included are pesticides including synthetic and biological-based pesticides and, in appropriate cases, herbicides.

In all cases, the final formulation should be clear to the naked eye. Preferably, the final formulation has a turbidity of less than 5 NTU, and more preferably less than 1 NTU.

In one preferred preparation, initially, an alkoxyated, typically ethoxyated, linear alcohol or alkoxyated/ethoxyated mixture of alcohols containing 9-11 carbons is added to one or more active ingredients followed by homogenizing. This is optionally followed by adding an ethoxyated linear alcohol of 11 carbons, and then homogenizing. This is then followed by adding a surfactant blend for microemulsion formation and homogenizing, and finally an anionic surfactant is added. The final composition is then homogenized. By using appropriate concentrations, the resultant forms a concentrated premix which then can be diluted for application to agricultural crops, or, if the active ingredient is a drug, may be used in undiluted form.

Thus, in the first step described above, mixtures of linear alcohols of suitable chain lengths with an appropriate degree of alkoxylation can be used. The proportions of C<sub>9</sub>, C<sub>10</sub> and C<sub>11</sub> alcohols can be variable, but typically, the mixture contains at least 25 percent of C<sub>9</sub> linear alcohols. Commercially available mixtures are typically ethoxyated to varying degrees, 2.5 moles of ethoxylation in the polymeric chain being preferred. Although ethoxylation is the most typical commercially available form, there is no theoretical reason that methoxylation or formation of polymeric ethers with higher chain alcohols could not

be used. Tomah Reserve, Inc. markets a series of linear alcohols trademarked Tomadol™. The Tomadol™ compositions vary in chain length of the alcohol and number of moles of ethoxylation. For compositions where the alcohol contains 11 carbons, 7 moles of ethoxylation per mole of alcohol appears optimal; 9 moles can result in a solid at room temperature. Further, in compositions which contain mixtures of 9-11 carbon alcohols, 2.5 moles ethoxylation per mole of alcohol appears to exhibit the best insect cuticle penetration.

In the second optional step, C<sub>11</sub> linear alcohols in ethoxylated form are also available. Typical levels of ethoxylation include 7 moles of monomer in the polymeric chain; as was the case with the ethoxylated mixture in the first step, other alkoxyated forms could be substituted.

In the third step, a surfactant blend for microemulsion formation is employed. Various commercial preparations are available, including those from Stepan Chemical Company, Winder, Georgia. Of seven microemulsion formulations produced by Stepan Chemical, the highly preferred formulation is commercially designated H306A. The company will produce this formulation on special order of 20,000 lb. batches.

In the fourth step, an anionic detergent or mixture of anionic detergents is used. Anionic surfactants, such as sodium lauryl sulfate and ammonium lauryl sulfate are known. Commercial mixtures are also available, although the variety of options available has decreased due to the introduction of siloxane-based surfactants now more commonly used in agriculture. However, anionic detergents are required in the compositions of the invention. One anionic surfactant currently marketed is Atsurf 1910 which, in its newer version Atsurf 1910 HF with a higher flash point, is less desirable than the version previously sold.

For agricultural uses, the composition is diluted 1:200, 1:500, 1:1000, or 1:2000 for application. The final concentration of the active ingredient is on the order of 200 ppm. The diluted composition must be clear and not turbid. Any dilution which does not appear clear to the naked eye should be rejected. It is preferable, however, that the turbidity of the final composition be less than 3 NTU, more preferably less than 2.5 NTU, and most preferably less than 1.0 NTU as measured in a turbidometer. The dilution may result in a satisfactorily clear solution without further agitation, or it may be necessary to stir, shake or vortex or otherwise agitate the dissolved components in order to affect clarity.

The following examples are intended to illustrate but not to limit the invention.

### Example 1

#### Preparation of Test Microemulsions

Four varying preparations of the pesticidal microemulsion were prepared as shown in Table 1 below. The preparation procedure was as follows: Treated and lipase associated sunflower oil was prepared by mixing 200 ml of a solution containing 200 mg of Validase 8000 (Valley Research Technologies, South Bend, IN) with 200 ml of Optimum™ sunflower oil, Optimum Quality Grains, Inc., Urbandale, IA. The resultant mixture was homogenized and incubated for 24 hours at 40°C. The mixture was then treated for 10 minutes at 95°C effecting a separation into an aqueous and lipid phase. The lipid phase was recovered.

A few large particles were formed which were removed by filtration through Whatman No. 1 filter paper. This mixture could be stored for as long as two years.

Final formulation of the concentrated pesticide required the addition of surfactants. The surfactants were added in the order described below; the ratio of the surfactants to the digested sunflower oil in various formulations is shown in Table 1. The order of addition is important.

To the treated sunflower oil was first added Tomadol 91-2.5, a Tomah Reserve Inc. product. This is a linear alcohol, ethoxylated C<sub>9</sub> - C<sub>11</sub> with 2.5 moles of ethoxylation. After the addition, homogenization was performed by stirring at 15,000 rpm maintaining the temperature at room temperature. This was followed by addition of Tomadol 1-7, another Tomah Reserve product which comprises linear alcohols of C<sub>11</sub> ethoxylated with 7 moles ethoxylation. The resulting mixture was again homogenized by stirring at 15,000 rpm. This was followed by addition of H306A, a product for microemulsion formation produced by the Stepan Chemical Company (Winder, GA). The composition is proprietary, but is commercially available. Following the addition of H306A, homogenization was performed again by stirring at 15,000 rpm. This was followed by addition of Atsurf 1910, a product of Uniqema (Wilmington, DE) which is a commercially available proprietary blend of anionic surfactants. The mixture was homogenized by stirring at 15,000 rpm. The final premix formulation was clear or cloudy as shown in Table 1 depending on the ratios of digested oils to surfactants.

The premix was diluted with water in a ratio to obtain at 200 ppm of the treated oil in the dilution and tested for ability to kill reniform nematodes in 30 inch x 2 inch soil

tubes. The percentages killed are given in Table 1. Also shown in Table 1 are the percentage kills of reniform nematodes when the premix was tested in water rather than soil at 200 ppm of the treated sunflower oil, with surfactants.

Table 1

Components	081499-3	081499-2	081599-1	081599-2
Treated Sunflower Oil	5	4	4	4
H306	12	12	12	12
1910	12	12	12	12
91-2.5	5	4	5	6
1-7	2	2	2	2
% DSO	13.9	11.8	11.4	11.1
Ratio DSO/Surfactant	1:6.2	1:7.5	1:7.75	1:8
Emulsion	CLOUDY	Clear	Clear	Clear
Water Test at 200 ppm %RENIFORM KILL	----	97%	98.5%	96.5%
SOIL TUBE TEST at 200 ppm digested oil	----	53%	100%	75%

As shown in Table 1, although all of the clear preparations were effective, preparation 081599-1 was the most effective in the soil tests.

### Example 2

#### Additional Formulations

Table 2 shows additional formulations of nematicidal materials.

For formulation 0510099-5, the hydrophobic/hydrophilic component is Dupont sunflower oil which has been treated with *Laetiporus* culture filtrate for 24 hours.

In formulation 122699-7, the hydrophobic/hydrophilic ingredient is Dupont sunflower oil which has been treated with Validase for 24 hours.

In formulation 122699-6, the hydrophobic/hydrophilic ingredient is the sugar alcohol ester Toximul SEE-340 which has been predissolved in aromatic 100.

In formulation 011500-1, the hydrophobic/hydrophilic component is safflower oil which is treated with a nine-months fermented *Laetiporus* culture.

Table 2

	051099-5	122699-7	122699-6	011500-1
Hydrophobic/Hydrophilic Treated Oil or Sugar Ester	3	3	3	3
H306A	12	14	20	12
1910	12	12	12	12
91-2.5	4	4	4	4
1-7	2	2	2	2
Aromatic 100	-	-	6	-
% Oil or SEE-340	9.09	8.57	6.4	9.09
NTU 200 ppm	0.7	1.2	2.2	
NTU 24 hrs later 200 ppm	0.7	1.2	1	
NTU 400 ppm			2.4	0.9
NTU 200 ppm			2.3	
Ratio ai/surfactant	1 to 10	1 to 10.7	1 to 14.7	1 to 10
500 ppm ai	5.5 ml/l	5.75 ml/l	7.83 ml/l	5.5 ml/l
400 ppm ai	4.4 ml/l	4.6 ml/l	6.26 ml/l	4.4 ml/l
200 ppm ai	2.2 ml/l	2.3 ml/l	3.13 ml/l	2.2 ml/l
100 ppm ai	1.1 ml/l	1.15 ml/l	1.57 ml/l	1.1 ml/l

ai = active ingredients

The formulation 12269-6 can also be represented as follows where the commercial sources of the components are noted.

#### All Surfactant Nematicide Formulation 122699-6

			ml
Stepan Company	Sorbitan trioleate	Toximul SEE-340	60
Stepan Company	Microemulsion blend	H306A	400
Unqema	Anionic surfactant	Atsurf 1910	240
Tomah Reserve	Linear Alcohol	91-2.5	80
Tomah Reserve	Linear Alcohol	1-7.0	40
Exxon Chemical Co.	Agriculture solvent	Aromatic 100	120
Total ml			940

500 ppm 2.0 NTU

250 ppm 1.5 NTU

In the foregoing formulations it has been shown that in the formulation treated with *Laetiporus fungi*, no hydrolyses of oleic acid esters appears to have occurred. In contrast,

in those formulations where the oil is treated with Validase, some hydrolysis appears to take place. While NTU for formulation 051099-5 and 122699-7 at 200 ppm was stable over 24 hours, NTU for 122699-6 at 200 ppm decreased over this time period.

The formulations prepared were subjected to soil tube testing which involves the dripping of a test solution from a separatory funnel on to an upright 2 inch diameter plastic pipe, 30 inches long. The pipe is cut at 18 inches from the top. This would represent 18 inches of soil depth as in the first section. The second section consists of 12 inches of soil. The bottom section has a bottom screen cover to prevent the soil from falling out. As soil is filled into the pipe sections, soil samples are placed on the side for the control nematode populations. Nematode populations are determined in the soil batch before soil testing commences. The two sections are joined by a rubber gasket secured by adjustable gasket clamps. The test solution is dripped into the top of the soil tube and the solution percolates through the soil tube and drips out. When the solution stops percolation, the soil tubes are disassembled and the soil is placed in a plastic bag for storage. Depending on the conditions, 1 to 7 days may elapse before the soil nematodes are extracted via the Baermann Funnel system. Each soil tube will have 2 controls and 2 treatments, a set of control and treatment for each section of the soil tube.

Some formulations to be tested may only show effectiveness in the top 18 inches, some may show no effectiveness in soil and some may show effectiveness throughout the 30 inch soil tube.

If a formulation shows effectiveness in the soil tube testing, the formulation will show effectiveness in pots and field tests as well.

The results of testing these formulations of Table 2 are shown in Tables 3 - 7.

Table 3

SOIL TUBE DATA		051099-5		NEMATODE COUNTS PER 50 ML OF SOIL						
Date	Formula	ppm active ingredient	Replicate	Soil Tube Section	Total	Reniform	Spiral	Rootknot	Others	
1/12/00	051099-5	125	1	510 1-Control	480	50	90	100	240	
				2-Control	460	60	100	150	150	
				3-Treated	70	0	20	30	20	
				4-Treated	310	10	30	100	170	
1/12/00	051099-5	125	2	511 1-Control	450	50	100	110	190	
				2-Control	560	60	100	190	210	
				3-Treated	50	0	10	10	30	
				4-Treated	420	20	30	50	320	
1/12/00	051099-5	125	3	512 1-Control	1180	80	250	300	550	
				2-Control	1240	90	260	280	610	
				3-Treated	40	0	10	10	20	
				4-Treated	370	30	50	90	200	

5 Section 1 and 3 are the control and treatment of the first 18 inches of soil.  
 Section 2 and 4 are the control and treatment of the next 12 inches of soil.  
 Formulations were dripped into the soil tube, approximately 1 liter in 2 hours.

Table 4

SOIL TUBE DATA					122699-7				
NEMATODE COUNTS PER 50 ML OF SOIL									
Date	Formula	ppm active ingredient	Replicate	Soil Tube Section	Total	Reniform	Spiral	Rootknot	Others
1/12/00	122699-7	400	1	504 1-Control	1350	120	310	400	520
				2-Control	2620	240	180	500	1700
				3-Treated	20	0	0	10	10
				4-Treated	360	20	50	200	90
1/12/00	122699-7	400	2	505 1-Control	1140	120	170	190	660
				2-Control	1040	40	60	170	770
				3-Treated	0	0	0	0	0
				4-Treated	140	0	0	20	120
1/12/00	122699-7	400	3	506 1-Control	760	40	60	110	550
				2-Control	640	40	50	120	430
				3-Treated	20	0	0	10	10
				4-Treated	50	0	0	30	20

Section 1 and 3 are the control and treatment of the first 18 inches of soil.

Section 2 and 4 are the control and treatment of the next 12 inches of soil.

Formulations were dripped into the soil tube, approximately 1 liter in 2 hours.



Table 5

SOIL TUBE DATA				122699-9					
NEMATODE COUNTS PER 50 ML OF SOIL									
Date	Formula	ppm active ingredient	Replicate	Soil Tube Section	Total	Reniform	Spiral	Rootknot	Others
1/12/00	122699-6	400	1	507 1-Control	810	60	70	150	530
				2-Control	1000	70	150	200	580
				3-Treated	0	0	0	0	0
				4-Treated	10	0	0	0	10
1/12/00	122699-6	400	2	508 1-Control	1240	70	120	130	920
				2-Control	1260	80	140	170	870
				3-Treated	0	0	0	0	0
				4-Treated	50	0	0	20	30
1/12/00	122699-6	400	3	509 1-Control	610	40	70	150	350
				2-Control	590	30	50	100	410
				3-Treated	10	0	0	0	10
				4-Treated	10	0	0	0	0

Section 1 and 3 are the control and treatment of the first 18 inches of soil.  
 Section 2 and 4 are the control and treatment of the next 12 inches of soil.  
 Formulations were dripped into the soil tube, approximately 1 liter in 2 hours.

Table 6

SOIL TUBE DATA				122699-6					
NEMATODE COUNTS PER 50 ML OF SOIL									
Date	Formula	ppm active ingredient	Replicate	Soil Tube Section	Total	Reniform	Spiral	Rootknot	Others
1/13/2000	122699-6	125	1	528 1-Control	950	50	60	100	740
				2-Control	1100	100	150	250	600
				3-Treated	50	10	10	20	10
				4-Treated	150	20	20	30	80
1/13/2000	122699-6	125	2	529 1-Control	1200	130	180	220	670
				2-Control	1800	150	250	300	1100
				3-Treated	50	10	10	10	20
				4-Treated	70	10	10	20	30
1/13/2000	122699-6	125	3	530 1-Control	1200	90	200	300	610
				2-Control	1100	110	180	250	560
				3-Treated	50	0	0	20	30
				4-Treated	220	20	40	60	100

5 Section 1 and 3 are the control and treatment of the first 18 inches of soil.  
 Section 2 and 4 are the control and treatment of the next 12 inches of soil.  
 Formulations were dripped into the soil tube, approximately 2 liters in 4 hours.

Table 7

SOIL TUBE DATA		011500-1		NEMATODE COUNTS PER 50 ML OF SOIL						
Date	Formula	ppm active ingredient	Replicate	Soil Tube Section	Total	Reniform	Spiral	Rootknot	Others	
1/17/2000	011500-1	500	1	531 1-Control	1120	120	250	280	470	
				2-Control	520	60	100	150	210	
				3-Treated	20	0	10	10	0	
				4-Treated	150	0	20	50	80	
1/17/2000	011500-1	500	2	532 1-Control	1500	100	250	350	800	
				2-Control	1100	90	150	250	610	
				3-Treated	20	0	0	0	20	
				4-Treated	30	0	10	10	10	
1/17/2000	011500-1	500	3	533 1-Control	1500	100	250	450	700	
				2-Control	1120	110	260	350	400	
				3-Treated	0	0	0	0	0	
				4-Treated	70	0	20	30	20	

5 Section 1 and 3 are the control and treatment of the first 18 inches of soil.

Section 2 and 4 are the control and treatment of the next 12 inches of soil.

Formulations were dripped into the soil tube, approximately 1 liter in 2 hours.

As shown in these tables, all of the formulations were effective as compared to controls. The counts of various nematodes are consistently smaller in both areas of the tubes than for controls. Formulation 122699-6 at 400 parts per million is particularly effective.

As this formulation is particularly successful, alternative representations of the data obtained with this formulation in soil tube tests at 125 and 400 parts per million of active ingredient, respectively, are presented in Tables 8 and 9. The active ingredient is sorbitan trioleate and the ppm ai represents parts per million of this component.

Table 9

SOIL TUBE DATA						122699-6				
Date	Formula	ppm ai	Repli- cate	Soil Tube	Section	Percentage Reduction in Nematode Count				
						Total	Reniform	Spiral	Rootknot	Others
1/13/2000	122699-6	125	1	528	1	95%	80%	83%	80%	99%
					2	86%	80%	87%	88%	87%
1/13/2000	122699-6	125	2	529	1	96%	92%	94%	95%	97%
					2	96%	93%	96%	93%	97%
1/13/2000	122699-6	125	3	530	1	96%	100%	100%	93%	95%
					2	80%	82%	78%	76%	82%
					1	95.7%	90.7%	92.3%	89.3%	97%
					2	87.3%	85%	87%	85.7%	88.7%

Section 1 is the first 18 inches of soil in the soil tube 2 inch diameter, 30 inches long. Section 2 is the lower 12 inches of the soil tube.

Formulations were dripped into the soil tube at a rate of 2 liters in 4 hours.

Table 10  
SOIL TUBE DATA 122699-6

Date	Formula	ppm ai	Repli- cate	Soil Tube	Section	Nematode Counts Per 50 MI Of Soil				
						Total	Reniform	Spiral	Rootknot	Others
12/27/1999	122699-6	400	1	507	1-Control	810	60	70	150	530
					2-Control	1000	70	150	200	580
					3-Treated	0	0	0	0	0
					4-Treated	10	0	0	0	10
12/27/1999	122699-6	400	2	508	1-Control	1240	70-	120	130	920
					2-Control	1260	80	140	170	870
					3-Treated	0	0	0	0	0
					4-Treated	50	0	0	20	30
12/27/1999	122699-6	400	3	509	1-Control	610	40	70	150	350
					2-Control	590	30	50	100	410
					3-Treated	10	0	0	0	10
					4-Treated	10	0	0	10	0

Section 1 and 3 are the control and treatment of the first 18 inches of soil in a 2 in pipe, 30 inches long.  
Section 2 and 4 are the control and treatment of the next 12 inches of soil in a 2 in pipe, 30 inches long.  
Formulations were dripped into the soil tube, approximately 1 liter in 2 hours.

### Example 3

#### Results of Field Tests

A field test evaluated the following formulations. The experimental design was as follows:

Randomized complete block 14 treatments X 5 replications (2 plant per replication)

**TREATMENTS****Treatments:**

AX	Control- water only
A0	Surfactant/Emulsifier A- no ABR
AC	Surfactant/Emulsifier A- Safflower Oil @ 500 ppm
A500	Surfactant/Emulsifier A(formulation 42399-1)- ABR @ 500 ppm
A2000	Surfactant/Emulsifier A(formulation 42399-1)- ABR @ 2000 ppm
BX	Control- water only
B0	Surfactant/Emulsifier B – no ABR
BC	Surfactant/Emulsifier B- Sunflower Oil @ 500 ppm
B500	Surfactant/Emulsifier B(formulation 051099-5)- ABR @ 500 ppm
B2000	Surfactant/Emulsifier B(formulation 051099-5)- ABR @ 2000 ppm
C0	Surfactant/Emulsifier D(formulation 052299-1)- ABR @ 2000 ppm
CC	Surfactant/Emulsifier C- Safflower Oil @ 500 ppm
C500	Surfactant/Emulsifier C(formulation 051799-1)- ABR @ 500 ppm
C2000	Surfactant/Emulsifier C(formulation 051799-1)- ABR @ 2000 ppm

As shown in the foregoing list, Surfactant/Emulsifier A, B, C, or D are based on the noted formulations. The noted formulations are composed of components listed as follows:

**Table 8****Treatment A series 042399-1****Parts**

*Laetiporus* treated Safflower Oil,  
24 hour

2

ICI's Atsurf 1910

5 (proprietary anionic surfactants)

ICI's Atplus 401

1 (proprietary surfactants)

OSI Specialties' SilWet L7280  
heptamethyl

2 (polyalkyleneoxide modified  
Trisiloxane)

Loveland Industries' Blend

1 (proprietary blend of surfactants)

Aldrich's Tween 85

1 (sorbitan esters)

70 NTU at 200 ppm active ingredient

---

**Treatment B 051099-5****Parts***Laetiporus* treated Optimum  
Sunflower Oil, 24 hours

3

H306A

12

1910

12

91-2.5

4

1-7.0

2

**0.7 NTU at 200 ppm active ingredient**

---

**Treatment CO 052299-1****Parts***Laetiporus* treated Optimum  
Sunflower Oil, 24 hours

3

H307AB Stepan Microemulsion Blend

12 (Discontinued formulation from Stepan)

1910

12

91-2.5

4

1-7.0

2

**0.7 NTU at 200 ppm active ingredient**

---

**Treatment C500 and C2000 051799-1****Parts***Laetiporus* treated optimum Sunflower  
Oil, 24 hours

3

H306

12

1910

12

91-2.5

4

1-7.0

2

**1.1 NTU at 200 ppm active ingredient**

30

Where - "ABR" is indicated, the hydrophobic/hydrophilic component is present. The notation "no ABR" indicates that this ingredient is absent as is any oil component whatsoever. Thus, for example, treatment A2000 represents formulation of 42399-1 as shown in Table 8, but diluted to 2000 parts per million with water. A500 indicates the same formulation, but diluted to 500 parts per million. AC represents the components of formulation 042399-1 without the *Laetiporus* treated safflower oil, but having substituted, in place of it, safflower oil without being treated. AO, which contains surfactants/emulsifier A - no ABR, contains all the components of the formulation shown except the treated safflower oil and no additional safflower oil is included. The remaining entries are interpreted in an analogous manner.

#### LAYOUT

Treatment Plots: 10 plants/ treatment

REP5	REP4	REP3	REP2	REP1	ROAD
AX 5	AX 4	AX 3	AX 2	AX 1	1
AO 5	AO 4	AO 3	AO 2	AO 1	2
AC 5	AC 4	AC 3	AC 2	AC 1	3
A500 5	A500 4	A500 3	A500 2	A500 1	4
A2000 5	A2000 4	A2000 3	A2000 2	A2000 1	5
C500 5	B500 4	BC 3	BO 2	BX 1	6
BX 5	B2000 4	B500 3	BC 2	BO 1	7
CC 5	CO 4	B2000 3	B500 2	BC 1	8
C2000 5	CC 4	CO 3	B2000 2	B500 1	9
B500 5	C500 4	CC 3	CO 2	B2000 1	10
BO 5	C2000 4	C500 3	CC 2	CO 1	11
CO 5	BX 4	C2000 1	C500 2	CC 1	12
BC 5	BO 4	BX 3	C2000 2	C500 1	13
B2000 5	BC 4	BO 3	BX 2	C2000 1	14

#### PROCEDURE

A first or second ratoon crop field that is not scheduled for another crop cycle and has a history of high nematode populations will be selected. Fourteen beds will be selected and walkways between the beds will be trimmed for approximately 25 feet. The first plant line in each bed will be used for testing with every other plant receiving the proper treatment. A soil sample for nematode will be taken pre application from the first,



third, fifth, seventh, ninth, eleventh, thirteenth, fifteenth, seventeenth and nineteenth plant in each treatment plant line. The first and third plant sample will be composited and recorded as replication 1, the fifth and seventh will be composited and recorded as replication 2. Samples will be composited in the same order so as to have 5 replications per treatment. Observations of plant color and vigor will be recorded and pictures will be taken pre application. Treatments will be applied to the second, fourth, sixth, eighth, tenth, twelfth, fourteenth, sixteenth, eighteenth and twentieth plants in each treatment plant line. Treatments will be applied in 2 liters of water, by hand, at the base of each plant using 2-liter coke bottles that have been punctured. Two liters per plant is approximately the amount that each plant would receive during a plantation practice Nematic application in a ½ acre-inch of water. At one month post application another soil sample for nematodes will be taken from the treated plants and observations of any changes in plant color and vigor (along with post application pictures) will be made. These soil samples will also be composited, with the sample from plants two and four recorded as replication 1, six and eight being replication 2 and so forth until 5 replications are collected.

#### RECORDS TO COLLECT

1. Map with location of test plot.
2. Records of dates and amounts of product and water applied.
3. Soil samples for nematodes pre application (un-treated plants).
4. Soil samples for nematodes 6 weeks post application (treated plants).
5. Phytotoxicity observations at 1,2 and 3 months after application.

#### RESULTS

##### Chronological data

AX, AO, AC, A500 and A2000 treatment applications took place on May 17, 1999. These applications were not randomized, with each treatment being applied to every even numbered plant (from 2 to 20) in the same plant line. The remainder of the treatments were applied on May 24, 1999 and were randomized. Pre application soil collection was conducted on May 13, 1999 and post application soil collection was

conducted July 12, 1999. Post application samples were split in half. The Maui Pine Ag Research lab and the University of Hawaii nematology lab each ran a set of samples using the elutriator and Baermann funnel techniques. This report will focus on the percent of reduction of nematode populations (pre application number of nematodes- post application number of nematodes/100), as pure numbers of nematodes can be misleading due to variation in the pre application counts.

#### Elutriator Results

Both Maui Pine and U.H. elutriator testing showed the B2000 treated plants to have the highest average percent reduction of all treatments. The Maui Pine data showed this percent reduction to average 63% and this treatment effect was statistically significantly larger than the average percent reduction in all other treatments except the B500 and C2000 treatments (percent reduction of 40% and 38%, respectively)(Table 1). The U.H. data showed the percent reduction in the B2000 treatments to average 86% and was statistically significantly larger than all the other treatments except the BO treatment (percent reduction of 54%)(Table 2).

#### Baermann Funnel Results

Both Maui Pine and U.H. Baermann funnel testing showed the B2000 treated plants to have the highest average percent reduction of all the treatments. The Maui Pine data showed this percent reduction to average 93% and was statistically significantly larger than the average percent reduction in the AO, AC, A500, A2000 and BX treatments. The C2000 treatments averaged a percent reduction of 92%, while the C500 and the CO treatments averaged 89%(Table 3). The U.H. data showed the B2000 percent reduction to average 97% and was statistically significantly larger than the AC, A500 and BX treatments. The CO and C500 treatments averaged a reduction of 95%(Table 4).

#### Phytotoxic Observations

No phytotoxic effect was observed in any of the plants treated during this trial. The last observation was conducted at more than 3 months after application.

## DISCUSSION

Both elutriator and Baermann funnel testing by the Maui Pine and U.H. labs indicate the B2000 formulation to be the most effective in controlling reniform nematodes. The B500 formulation showed good efficacy in both U.H. and Maui Pine elutriator testing but was not as effective as the B2000 formulation, indicating increasing efficacy at higher concentrations of ABR. The C2000 formulation consistently was among the most effective treatments and was generally followed by the C500 formulation, indicating an effective formulation (but not as effective as the B formulation) which was also more effective at the higher concentration of ABR. The CO treatment did show good efficacy, but mostly in the Baermann funnel data.

## CONCLUSION

The data indicate a level of control of reniform nematodes in the B, C and D emulsifier/surfactant formulations, with the B being the most effective. Higher concentrations of ABR generally gave a higher level of control and did not appear to have a phytotoxic effect on the plants treated in this trial.

### Example 4

#### Additional Nematicide Formulation

An additional formulation is prepared in a manner similar to that set forth in Example 1 except that a crude cheese extract is used in place of Validase, safflower oil is the oil treated, and white pepper is added as an antimicrobial agent. The adjuvant formulation of detergents and surfactants is as described. The formulation is preferably made with Munster or blue cheese.

## CLAIMS

1. A complex consisting essentially of
  - (a) a lipase associated with a least one C<sub>16</sub>-C<sub>20</sub> monounsaturated fatty acid or an ester thereof;
  - (b) an anionic surfactant blend;
  - (c) a microemulsion surfactant blend; and
  - (d) optionally, ethoxylated linear alcohols of C<sub>8</sub>-C<sub>11</sub>.
2. The complex of claim 1 wherein said ester is a monoglyceride, a triglyceride or a diglyceride.
3. The complex of claim 1 wherein the monounsaturated C<sub>16</sub>-C<sub>20</sub> fatty acid is an oleic acid.
4. The complex of claim 1 wherein said fatty acid or ester is derived from a vegetable oil.
5. The complex of claim 1 wherein said lipase is a fungal lipase.
6. The complex of claim 4 wherein the fungal lipase is derived from a *Laetiporus*, *Ganoderma*, *Mucor*, *Rhizopus*, *Penicillium*, *Candida* or *Aspergillus*.
7. The complex of claim 6 wherein said fungal lipase is derived from *Laetiporus*.
8. The complex of claim 1 wherein said lipase is derived from a bacterium.
9. The complex of claim 7 wherein the lipase is derived from *Pseudomonas*, *Rhizobium* or *Chromobacterium*.
10. The complex of claim 1 wherein said lipase is provided in purified form.

11. The complex of claim 5 wherein the lipase is provided as a fungal culture filtrate.
12. The complex of claim 1 wherein at least one surfactant comprises polyalkoxy chains.
13. The complex of claim 12 wherein said polyalkoxy chains are polyethoxy chains.
14. The complex of claim 1 contained in a clear aqueous emulsion.
15. The complex of claim 14 wherein said emulsion has a turbidity less than 1 NTU.
16. The complex of claim 1 wherein the ratio of component (a) to surfactant is about 1:6.5-1:8.5.
17. The complex of claim 16 wherein the ratio of component (a) to surfactant is 1:7.75.
18. A pesticide composition comprising the complex of claim 1 in a clear aqueous emulsion.
19. A complex consisting essentially of
  - (a) a saccharide, esterified to at least one monounsaturated C<sub>16</sub>-C<sub>20</sub> fatty acid;
  - (b) an anionic surfactant blend;
  - (c) a microemulsion surfactant blend; and
  - (d) optionally, ethoxylated linear alcohols of C<sub>8</sub>-C<sub>11</sub>.
20. The complex of claim 19 wherein said moiety of (a) further comprises polyalkoxy chains.

21. The complex of claim 20 wherein said polyalkoxy chains are polyethoxy chains.
22. The complex of claim 19 wherein said fatty acid is oleic acid.
23. The complex of claim 19 wherein said saccharide is sorbitol.
24. The complex of claim 19 wherein said fatty acid is derived from a vegetable oil.
25. The complex of claim 19 contained in a clear aqueous emulsion.
26. The complex of claim 25 wherein said emulsion has a turbidity less than 1 NTU.
27. The complex of claim 19 wherein the ratio of component (a) to surfactant is about 1:6.5-1:8.5.
28. The complex of claim 27 wherein the ratio of component (a) to surfactant is 1:7.75.
29. A pesticide composition comprising the complex of claim 19 in a clear aqueous emulsion.
30. A pesticide premix comprising the complex of claim 1.
31. A pesticide premix comprising the complex of claim 19.
32. A pesticide composition comprising a diluted form of the premix of claim 30.

33. A pesticide composition comprising a diluted form of the premix of claim 31.
34. A method to control agricultural pests wich method comprises applying the composition of claim 32 to an area in which such control is desired.
35. A method to control agricultural pests wich method comprises applying the composition of claim 33 to an area in which such control is desired.
36. A method to prepare a pesticide composition which method comprises preparing a mixture of an aqueous lipase solution with an oil wherein said oil consists essentially of triglycerides composed of C<sub>16</sub>-C<sub>20</sub> monounsaturated fatty acids;  
homogenizing said mixture to obtain an emulsion and incubating said emulsion for a time and at a temperature sufficient to associate said lipase with said oil;  
denaturing said emulsion under conditions which result in separation of the oil and water in said emulsion;  
removing solid particles if solid particles are present;  
adding surfactants to the separated emulsion to obtain a resultant; and  
homogenizing the resultant to obtain a clear aqueous microemulsion.
37. The method of claim 36 wherein said lipase is derived from a fungus.
38. The method of claim 37 wherein said fungus is *Laetiporus sulphureus*, *Ganoderma*, *Pleurotus*, *Aspergillus*, *Candida*, *Mucor*, *Rhizopus* or *Penicillium*.
39. The method of claim 36 wherein said lipase is derived from a bacterium.
40. The method of claim 39 wherein said bacterium is *Pseudomonas spp* or *Chromobacterium spp*.

41. The method of claim 36 wherein said lipase is derived from a plant or animal.
42. The method of claim 36 wherein the lipase is supplied as a fungal culture filtrate.
43. The method of claim 36 wherein said step of adding surfactant comprises adding four different surfactants.
44. The method of claim 43 wherein said adding surfactant comprises first adding an ethoxylated linear alcohol containing 9-11C; followed by adding an ethoxylated linear alcohol of 11C; followed by adding a surfactant blend for microemulsion formation; followed by adding an anionic surfactant or anionic surfactant blend; wherein each adding step is followed by an homogenizing step.
45. A method to prepare a pesticide composition which method comprises preparing an aqueous solution of a moiety having a hydrophilic core covalently bound to the backbone of at least one monounsaturated C<sub>16</sub>-C<sub>20</sub> fatty acid, adding surfactants to the solution to obtain a resultant, and homogenizing the resultant to obtain a clear aqueous microemulsion.
46. The method of claim 45 wherein said step of adding surfactant comprises adding four different surfactants.
47. The method of claim 46 wherein said adding surfactant comprises first adding an ethoxylated linear alcohol containing 9-11C; followed by adding an ethoxylated linear alcohol of 11C; followed by adding a surfactant blend for microemulsion formation; followed by adding an anionic surfactant or anionic surfactant blend; wherein each adding step is followed by an homogenizing step.



48. A dispersant/penetrant composition which composition consists essentially of

- (a) at least one linear alcohol of 7-12C which is polyalkoxylated;
- (b) at least one microemulsion enhancing component; and
- (c) at least one anionic detergent.

49. The composition of claim 1 wherein the polyalkoxylated linear alcohol has an alkoxyene:alcohol ratio of 8:1 to 2:1.

50. The composition of claim 49 wherein said ratio is 2.5:1.

51. The composition of claim 50 which further contains at least one C<sub>11</sub> alcohol with a polyalkoxylation ratio of 7:1.

52. The composition of claim 48 wherein said microemulsion-enhancing component is a blend of nonionic surfactants.

53. The composition of claim 48 wherein the anionic detergent comprises a salt of lauryl sulfate.

54. The composition of claim 48 wherein the ratio of components (a):(b):(c) is about 1-5(a): 9-15(b): 9-15(c).

55. The composition of claim 48 which is selected from the group consisting of:

- 5 parts 91-2.5, 2 parts 1-7, 12 parts H306, 12 parts 1910;
- 4 parts 91-2.5, 2 parts 1-7, 12 parts H306, 12 parts 1910;
- 6 parts 91-2.5, 2 parts 1-7, 12 parts H306, 12 parts 1910;
- 4 parts 91-2.5, 2 parts 1-7, 14 parts H306A, 12 parts 1910;
- 4 parts 91-2.5, 2 parts 1-7, 20 parts H306A, 12 parts 1910;

56. A formulation for pharmaceutical use which formulation comprises a pharmaceutically active compound and the composition of claim 48.

57. A method to administer a pharmaceutically active compound to a subject which method comprises topically applying to said subject the formulation of claim 56.

58. A formulation for agricultural use which formulation comprises an active ingredient at least one plant nutrient or at least one herbicide or at least one pesticide and the composition of claim 48.

59. The formulation of claim 58 which further comprises water as a diluent.

60. The formulation of claim 58 wherein said active ingredient is a plant nutrient.

61. A method to enhance the growth of a plant which method comprises applying topically to said plant the formulation of claim 60.

62. The formulation of claim 58 wherein said active ingredient is a pesticide.

63. A method to diminish the pest burden of a plant which method comprises applying topically to said plant an effective amount of the formulation of claim 62.

64. The formulation of claim 58 wherein said active ingredient is an herbicide.

65. A method for controlling weeds in a target area which method comprises applying to said target area an effective amount of the formulation of claim 64.